

***Pfiesteria* Surveillance, Monitoring and Research in Virginia**

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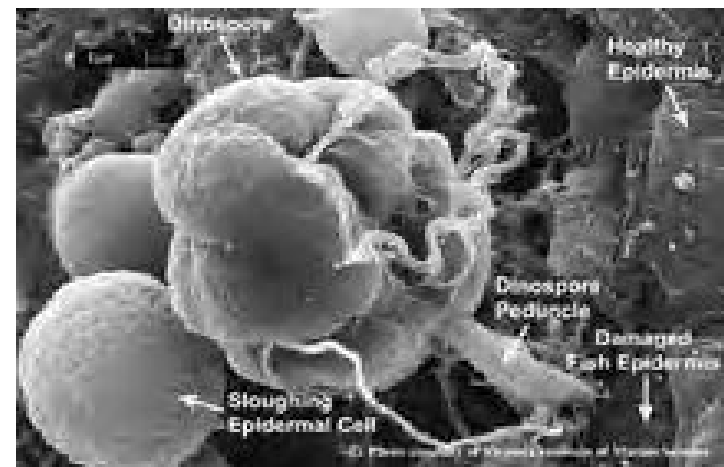


Pfiesteria piscicida



*Pfiesteria
shumwayae*

Pyrrophycophyta -
dinoflagellates



Harmful Algae



- ⌘ Other harmful dinoflagellates:
- ⌘ *Karenia brevis* – neurotoxic shellfish poisoning
- ⌘ *Prorocentrum* spp. *Dinophysis* spp. – diarrhetic shellfish poisoning
- ⌘ *Alexandrium*, *Gymnodinium*, *Pyrodinium* –
 - ☒ Paralytic shellfish poisoning

Early Investigations of *Pfiesteria* and Human Health



⌘ North Carolina Laboratory Reports

- ☒ Workers exposed to aerosols from toxic cultures of *Pfiesteria piscicida*
 - ☒ Faulty air handling system involved
- ☒ Variety of non-specific symptoms that cleared when exposure was controlled

Early Investigations of Pfiesteria and Human Health



⌘ North Carolina Laboratory Reports (*con't.*)

- ☒ Some physician and some self reporting, but no controlled studies
- ☒ Skin lesions, respiratory problems, gastrointestinal symptoms, disorientation, immunologic compromise, short term memory loss and/or severe cognitive impairment

Early Investigations of Pfiesteria and Human Health



⌘ Anecdotal information that North Carolina waterman had similar symptoms

☐ 1995 North Carolina Health Department review of persons exposed to fish kills - results inconclusive

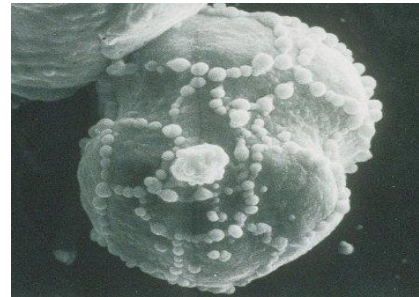
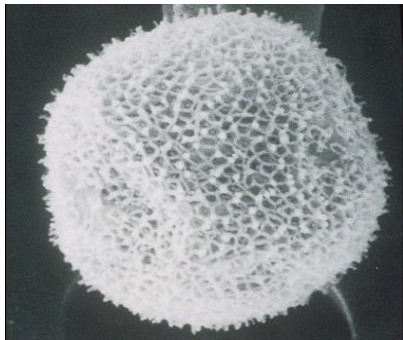
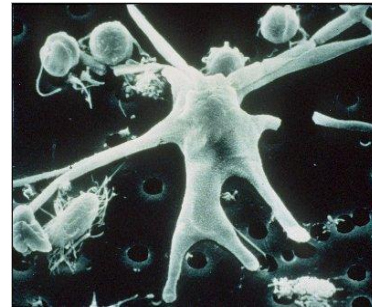
24 life forms

[illegible]

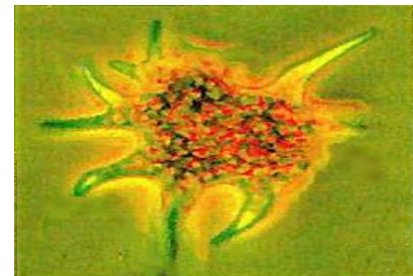
cyst



amoeboid



zoospore



FROM *THE NEW YORK TIMES*,
AUGUST 27, 1996:

By WILLIAM J. BROAD

LIKE something out of a horror movie, the cell from hell attacks its victims in gruesome ways, frequently changing its body form with lightning speed. The unicellular animal, called *Pfiesteria piscicida*, has at least 24 guises it can assume in the course of its lifetime. It can also masquerade as a plant or lie dormant for years in the absence of suitable prey.

Armed with a voracious appetite and vast reproductive powers, the microscopic animal moves through coastal waters to kill fish and shellfish by the millions and to poison anglers and others, producing pain, narcosis, disorientation, nausea, fatigue, vomiting, memory loss, immune failure and personality changes. Its toxins are so deadly that people who merely inhale its vapors can be badly hurt.

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Second Fish Kill Strikes Pocomoke

Toxic Microbe Suspected in Death of 2,000 in Virginia Waters

By Todd Shields

Washington Post Staff Writer

Wednesday, August 27, 1997; Page B03

The Washington Post

The Eastern Shore's Pocomoke River suffered a second fish kill yesterday as an estimated 2,000 fish died near the spot where 10,000 or more fish died earlier this month, Virginia officials said.

Yesterday's fish deaths took place in Virginia waters across the river's mouth from Maryland waters where the toxic microbe *Pfiesteria piscicida* was active during a four-day fish kill less than three weeks ago, said Jack Travelstead, chief of fisheries management for the Virginia Marine Resources Commission.

Virginia state workers took water and fish samples that will be sent to laboratories and analyzed for the presence of *Pfiesteria*, Travelstead said. He said most of the fish dying yesterday bore sores, a symptom seen in previous attacks by *Pfiesteria*.

**Cells From Hell -
update
Thursday, 22
February 2001**

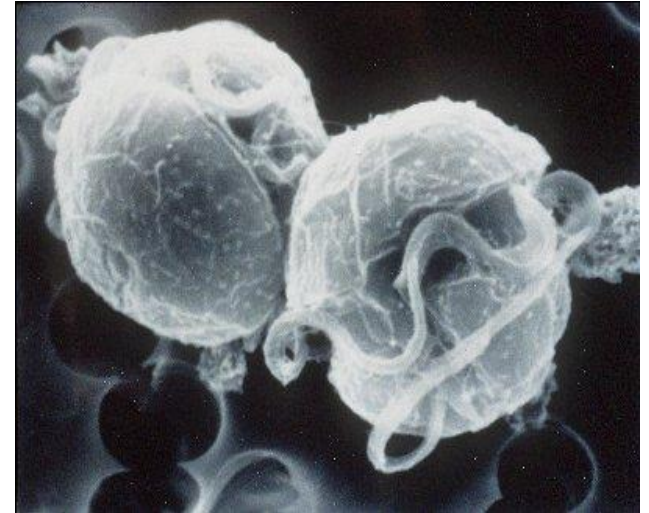


Shape-shifting
Pfiesteria

Last year we brought you the story of the *Pfiesteria*, a bizarre and highly toxic marine alga that can shape-shift from docile grazer to voracious carnivore in minutes.

VA Pfiesteria Task Force

- ⌘ DEQ – fish kills, water monitoring (Chair)
- ⌘ VDH - Public relations/health advisories
- ⌘ VCU - cohort study
- ⌘ VIMS - fish sampling
- ⌘ ODU - phytoplankton
- ⌘ VMRC – past-Chair of TF
 - ☐ enforce closures



Pfiesteria Surveillance, Monitoring and Research in Virginia



**Cooperative Agreement with CDC
To study Pfiesteria in Virginia
waters and determine the
potential for human health effects
began in 1997**

Terminology



- ⌘ EAS - Estuary Associated Syndrome
- ⌘ PEAS – possible Estuary Associated Syndrome

Estuary Associated Syndrome



- ⌘ Case definition:
- ⌘ Exposure to estuarine water,
- ⌘ Memory loss and/or confusion or
- ⌘ 3 or more of the following: headache, skin rash, burning skin, eye irritation, upper respiratory irritation, muscle cramps, GI symptoms for 2 or more weeks (except for skin rash and burning skin), and
- ⌘ A health care provider cannot identify another cause for the symptoms

Pfiesteria Surveillance, Monitoring and Research in Virginia



- ⌘ Passive surveillance of general population
- ⌘ Prospective cohort study to determine human health effects
- ⌘ Environmental monitoring

Passive Surveillance in Virginia

- ⌘ Hot line for obtaining information or to report health symptoms estb'd Sept. 1997 (1-888-238-6154)
- ⌘ Passive surveillance through local health departments or directly to DZEE
 - ☑ Service Coordinators in local health departments assisted with referrals, provided information and guidance on common diseases to rule out and arranged for special testing when indicated

PEAS Surveillance in Virginia

Year	Questions	UHC	Possible Exp.	Total calls
<i>1997</i>	<i>114</i>	<i>34</i>	<i>2</i>	<i>157</i>
1998	51	10	0	61
1999	9	3	0	12
2000	2	2	0	4
2001	0	0	0	0
2002	0	1	0	1
2003	0	0	0	0

Early Investigations of Pfiesteria and Human Health



⌘ VDH Passive Surveillance

- ⌘ 9 - further examination recommended
 - ⊗ 5 - volunteered for examinations (internist, neurologist, dermatologist if indicated and laboratory tests)
 - ⊗ 4 - full battery of neurocognitive testing
 - ⊗ 3 - Magnetic Resonance Imaging (MRI)

Early Investigations of Pfiesteria and Human Health



- ⏏ Two persons had mild problems with attention, memory and verbal learning tasks
 - ⏏ Unexplained by other conditions
 - ⏏ Both were on Pocomoke River during fish kill
 - ⏏ Abnormalities on MRI did not appear related
- ⏏ 1 Neurologic + dermatologic
- ⏏ 1 Neurologic

PEAS Surveillance in Virginia

Year	Questions	UHC	Possible Exp.	Total calls
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2003	0	0	0	0

PEAS Surveillance in Virginia

- ⌘ Contact with physicians (97,98,99,02)
- ⌘ Brochures
- ⌘ Fact sheets
- ⌘ Press releases and press conferences
- ⌘ Pfiesteria Task Force meetings
- ⌘ Web Page <http://www.vdhweb/WHC>
- ⌘ Virginia Epidemiology Bulletin

PEAS Surveillance in Virginia

- ⌘ Virginia Pfiesteria Cohort Study – Elizabeth E. Turf, Ph.D. VCU-SERL
- ⌘ 5 year study
- ⌘ 100 participants
- ⌘ location, duration of estuarine water exposure - 2x/month
- ⌘ 2x/year thorough physical, neurological, neuropsychological examinations

Active Surveillance in Virginia

- ⌘ Active surveillance triggered by:
 - ⌘ 1. fish kills (+PCR probe)
 - ⌘ 2. Pfiesteria or PLO blooms, (+PCR probe) or,
 - ⌘ 3. Disease clusters or outbreaks in general population
 - ⌘ 4. Neurocognitive symptoms in the cohort (3 or more)

Pfiesteria Monitoring in Virginia



1. Intended to determine an association between exposure to estuarine waters containing Pfiesteria, or PLOs, and Pfiesteria-related illness
2. To determine the presence and abundance of Pfiesteria and PLOs in VA waters, and
3. To characterize the variables that maintain and promote Pfiesteria and PLOs in VA estuaries.

Terminology



- ⌘ PCO = *Pfiesteria* Complex Organisms
- ⌘ PLO = *Pfiesteria* -like organisms
- ⌘ Approximately 10 organisms have been observed that are closely related to *Pfiesteria piscicida*, including *Pfiesteria shumwayae*, *Cryptoperidiniopsis* spp., "Lucy,"

Pfiesteria Monitoring Programs in Virginia

⌘ Water monitoring programs

⌘ Cohort sites 2x/month

⌘ DEQ water quality sites 1x/month

⌘ 50-105 shellfish sanitation stations
1x/month

⌘ Others: Great Wicomico River Study (VIMS), algal bloom, fish kill, Ches Bay collections

Pfiesteria Monitoring in Virginia



⌘ Water and sediment samples

- ☒ Phytoplankton identification –Pfiesteria spp., and PLOs
 - ☒ Light microscopy - PLO counts
 - ☒ SEM
 - ☒ PCR probes (2000, 2001, 2002)
- ☒ Cohort (2x), shellfish(1x), fish kill and algal bloom sites (event)

Pfiesteria Monitoring in Virginia



⌘ Water and sediment samples

☑ Toxic bioassays

- ☑ High PLOs

- ☑ Potentially toxic PLOs

- ☑ Algal bloom and fish kill sites

- ☑ Positive PCR probes



Level 3 protection
in the lab



Level B
protection in the
field

Water Quality Parameters

Water Temp, C
Dissolved Oxygen
pH
Conductivity
Salinity
Secchi depth
Total Nitrate Nitrogen
Total Nitrite Nitrogen
Total Ammonia
Nitrogen
Total Orthophosphate
Total Phosphorus
Total Kjeldahl
Nitrogen
Total Suspended Solids
BOD

Total Organic Carbon
Particulate Nitrogen
Particulate Phosphorous
Urea (99)
Dissolved Silica
Total Dissolved Nitrogen
Total Diss. Phosphorus
Diss. Orthophosphate
Chlorophyll a, corrected
Phaeophytin a
Preserved PLO, Lugols
Unpreserved PLO
Sediment for Culture
Genetic probe analysis
(99,00,01)

Pfiesteria Monitoring Programs in Virginia



⌘ Sediment collections:

⌘ Cohort sites 2x/month

⌘ DEQ water quality sites 1x/month

⌘ 50-105 shellfish sanitation stations
1x/season

⌘ Others: Great Wicomico River Study (VIMS), algal bloom, fish kill, Ches Bay collections

Pfiesteria Monitoring in Virginia



⌘ Fish Sampling

- ☒ Cohort sites 2x/month 1998-2000, 2002
1x/month 2001
- ☒ VIMS trawl survey
- ☒ Great Wicomico River Study (VIMS)

⌘ Fish pathology on fish w/ lesions

Molecular Monitoring for *Pfiesteria* in Virginia



- “Lucy”- like PLOs (most abundant and prevalent of the PLOs in VA)
 - 195 samples
 - Up to 212 cells/ml
- *P. shumwayae*
 - 5 samples
 - Up to 2 cells/ml
- *P. piscicida*
 - 7 samples
 - Up to 3 cell/ml
- *Aphanomyces invadans*
 - 19 samples
 - Less than 1 cell/ml

Molecular Monitoring for Pfiesteria in Virginia



⌘ 1998-1999

☑ no positive id from Rublee or Oldach

⌘ 1997 Mosquito Creek - Rublee

⌘ 1995 York River - Burkholder

Pfiesteria Monitoring Programs in Virginia



⌘ Fish monitoring programs

- ⌘ Cohort sites 2x/month
- ⌘ VIMS trawl survey (since 1956)
- ⌘ Great Wicomico River Study (VIMS)

Pfiesteria Monitoring in Virginia

⌘ Water and sediment sample collection:

⌘ Division of Shellfish Sanitation (DSS)

⌘ Department of Environmental Quality (DEQ)



Pfiesteria Monitoring in Virginia

⌘ Water and sediment sample analysis:

⌘ Old Dominion University (ODU)

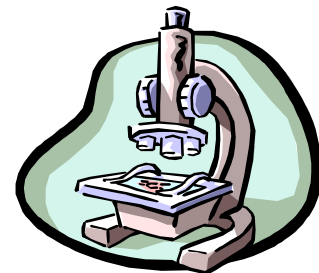
☒ Microscopy, toxic bioassays

⌘ Virginia Institute of Marine Sciences (VIMS)

☒ PCR analysis

⌘ Division of Consolidated Laboratory Services (DCLS)

☒ Water chemistry



Pfiesteria Monitoring in Virginia - Results

- ⌘ PLO abundance and water quality parameters –correlations varied for river grouping
 - ⌘ 13 parameters : salinity, secchi, temp, DO, chl a, tP, pP, TKN, tdN, pN, NH₄, silica, pC
- ⌘ PLO abundance 2002 much lower than previous years - 31% of samples v.
2001:62% 2000:61% 1999:50%
1998:45%
- ⌘ preliminary results for 2004 – earliest showing of PLO in VA since began sampling

Pfiesteria Monitoring in Virginia

⌘ Fish sampling and analysis:

☐ Virginia Institute of Marine Sciences (VIMS)



*Menhaden lesions containing the fungal pathogen
Photo courtesy of USGS Leetown Science Center*

Pfiesteria Monitoring in Virginia

⌘ Fish kill & algal bloom response:

☑ Department of Environmental Quality (DEQ)



VA Sites with *P. piscicida* and/or *P. shumwayae* Aug.-Oct. 2000, May-Oct. 2001, May-Nov. 2002

2000

53 samples
38 sites
17 + *P. piscicida* samples / 9 + sites
10 + *P. shumwayae* samples / 6 + sites

2001

385 samples
38 sites
6 + *P. piscicida* samples / 5 + sites
8 + *P. shumwayae* samples / 7 + sites

2002

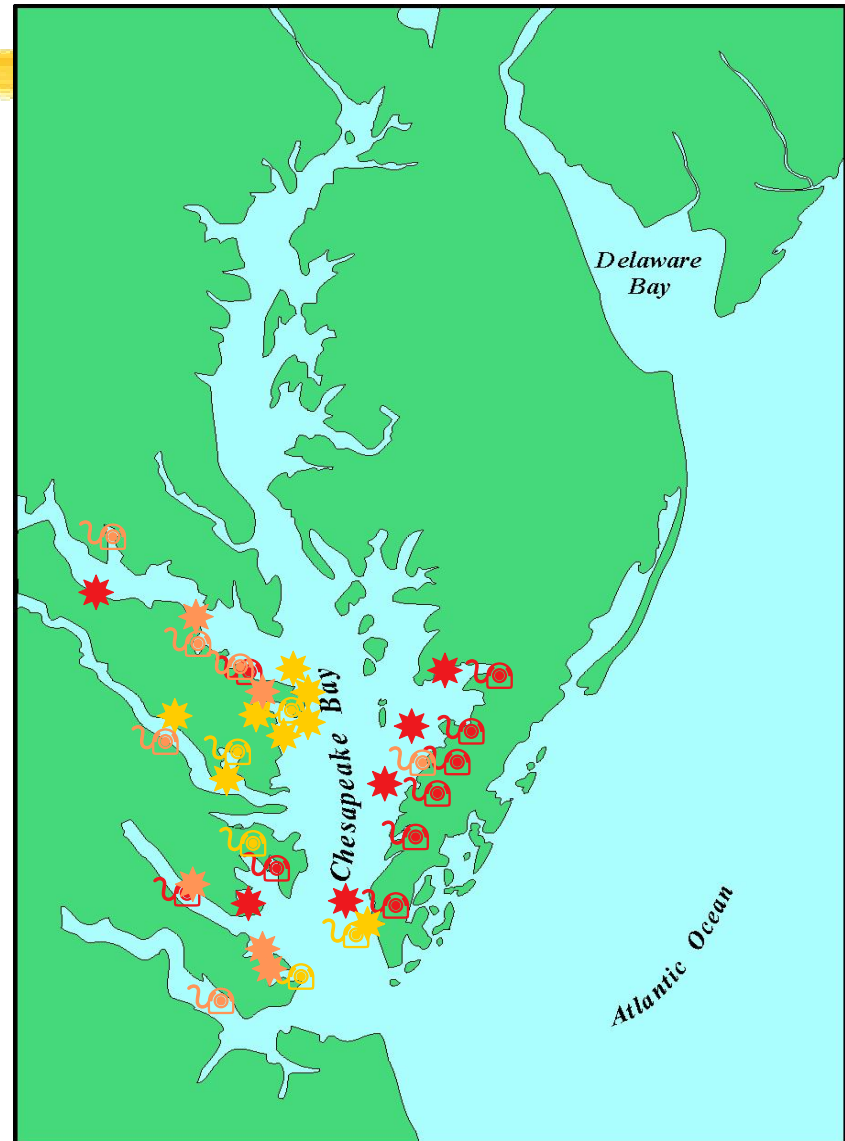
305 samples
38 sites
7 + *P. piscicida* samples / 6 + sites
5 + *P. shumwayae* samples / 5 + sites

'00 '01 '02



 *P. piscicida*


 *P. shumwayae*



Areas of Controversy



⌘ Pfiesteria Life-cycle:

- ☒ ODU – 24 stages, including amoeboid
- ☒ VIMS – no amoeboid stage, zoospore and cyst stages, toxic and non-toxic strains

⌘ Toxicity of Pfiesteria:

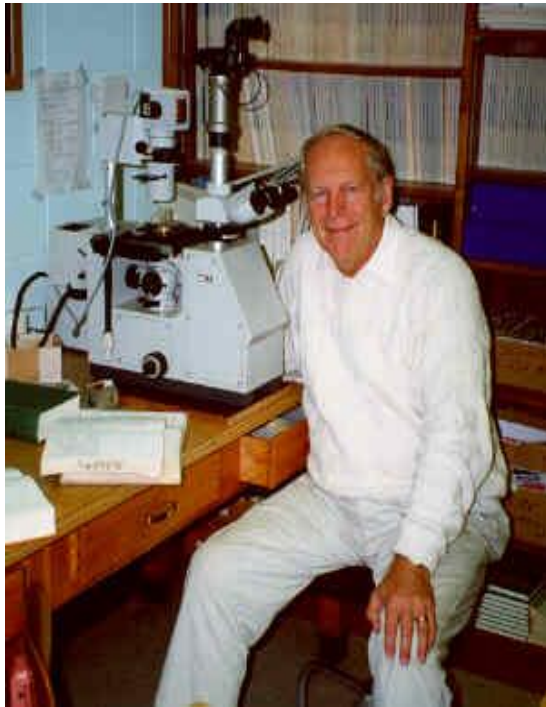
- ☒ ODU – toxin
- ☒ VIMS – no toxin, direct predation

⌘ Lesions on fish:

- ☒ ODU – caused by Pfiesteria toxin
- ☒ VIMS – caused by fungus: *Aphanomyces*

Pfiesteria Research in Virginia

- ⌘ Toxic Phytoplankton Identification - ODU
- ⌘ Toxic cell culture – ODU, VIMS
- ⌘ Toxic bioassays on mammals
 - ⌘ VIMS – mice
 - ⌘ ODU – rats
- ⌘ Toxic bioassays on fish- ODU, VIMS
- ⌘ Refinement of “tools” – PCR R&D
- ⌘ Other research: predation studies, retention in shellfish, toxicity, toxin isolation



Harold Marshall

**Old Dominion
University- ODU**



Perry Duncan



Andrew Gordon



Kim Reece



Larry Haas

**Virginia Institute
of Marine
Science -VIMS**



Jeff Shields



Wolf Vogelbein

Pfiesteria Monitoring & Research in Virginia



⌘ Refinements to toxic bioassays VIMS

- ☑ Using fish and shellfish tissue as a “lure” for field isolates of *Pfiesteria* spp.
- ☑ Mummichogs, sheepshead minnows
- ☑ Fractionation studies

Pfiesteria Monitoring & Research in Virginia

⌘ Toxic bioassays on mammals

⌘ VIMS – mice

⌘ ODU - rats



Pfiesteria Monitoring & Research in Virginia



- ⌘ Filter feeders as monitoring tools

- ⌘ VIMS

- ⌘ Menhaden, oysters

- ⌘ Passive vectors in Pfiesteria life cycle?

- ⌘ "Sentinel" oysters and menhaden?

Pfiesteria Monitoring & Research in Virginia



- ⌘ Toxic bioassays on fish

 - ⌘ VIMS, ODU

 - ⌘ Determining toxicity of PLOs at fish kills, algal blooms, fish lesion events since 1999

Pfiesteria Monitoring & Research in Virginia

⌘ Toxic cell culture

⌘ ODU and VIMS maintain toxin producing strains of *P. piscicida* and *P. shumwayae*

⌘ Used in bioassays and toxin related studies



Pfiesteria Monitoring in Virginia - Results

⌘ Predominant PLO

- ☒ 2004 – *Prorocentrum minimum*, *Heterocapsa triquetra*, *Katodinium rotundatum*, *Gymnodinium* spp.,
- ☒ 2002 – *Dinophysis acuminata*
- ☒ 2001 – *Gymnodinium*, *Gyrodinium*, *Heterocapsa*, *Cryptoperidiniopsis*, *Amphidinium*
- ☒ 2000 – *Cryptoperidiniopsis*, *Gyrodinium*, *Gymnodinium*
- ☒ 1999 – *Cryptoperidiniopsis*, *Gymnodinium*
- ☒ 1998 - *Cryptoperidiniopsis*